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08

An Optimized Interdigitated Array Microelectrode (IDA) Based Immunosensor for Detection of Avian Influenza Viruses

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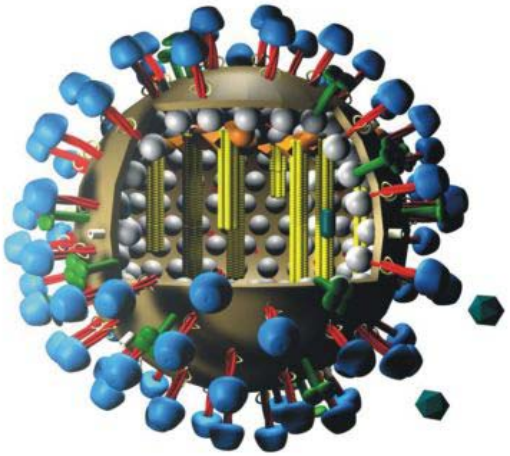
Introduction

- *Avian influenza (AI)*
  - A threat towards not only wild birds, but also poultry and human
  - The Cumulative number of confirmed human cases of avian influenza A/H5N1 reported to WHO has reached 370, with 235 deaths by March 4th, 2008
- *Avian influenza viruses (AIV):*
  - *Influenza* A viruses
  - Highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (IPAI)
  - The HPAI Influenza virus A/H5N1
    - Responsible for human infection cases reported recently



http://commons.wikimedia.org/wiki/Image:Flu_und_legende_color_c.jpg

Introduction

- *Identification of AIV*
  - Control the spread, reduce the potentially exposure of human to the viruses and minimize the economic loss
- *Current detection methods and limitations*
  - Viral culture: long detection time
  - RT-PCR: complex sample preparation procedures, and false positives
  - ELISA: limited specificity and sensitivity
- *IDA based impedance measurement*
  - higher sensitivity, less detection time, less sample volume needed, high signal:noise ratio

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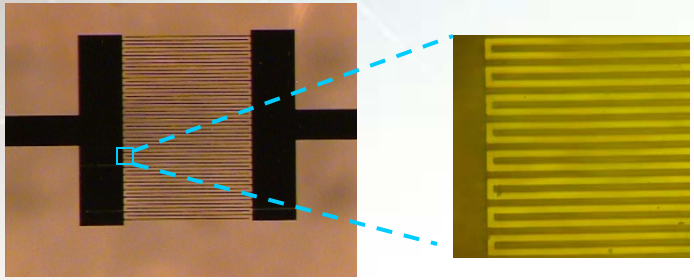
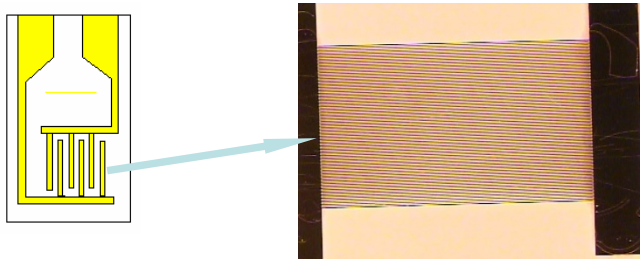
Materials and methods

- *IDA chips*



A 25-10-10 chip: 25 pairs of fingers, 10 µm the width of the fingers and the spaces between them



A 50-15-15 chip from ABTech Scientific Inc.

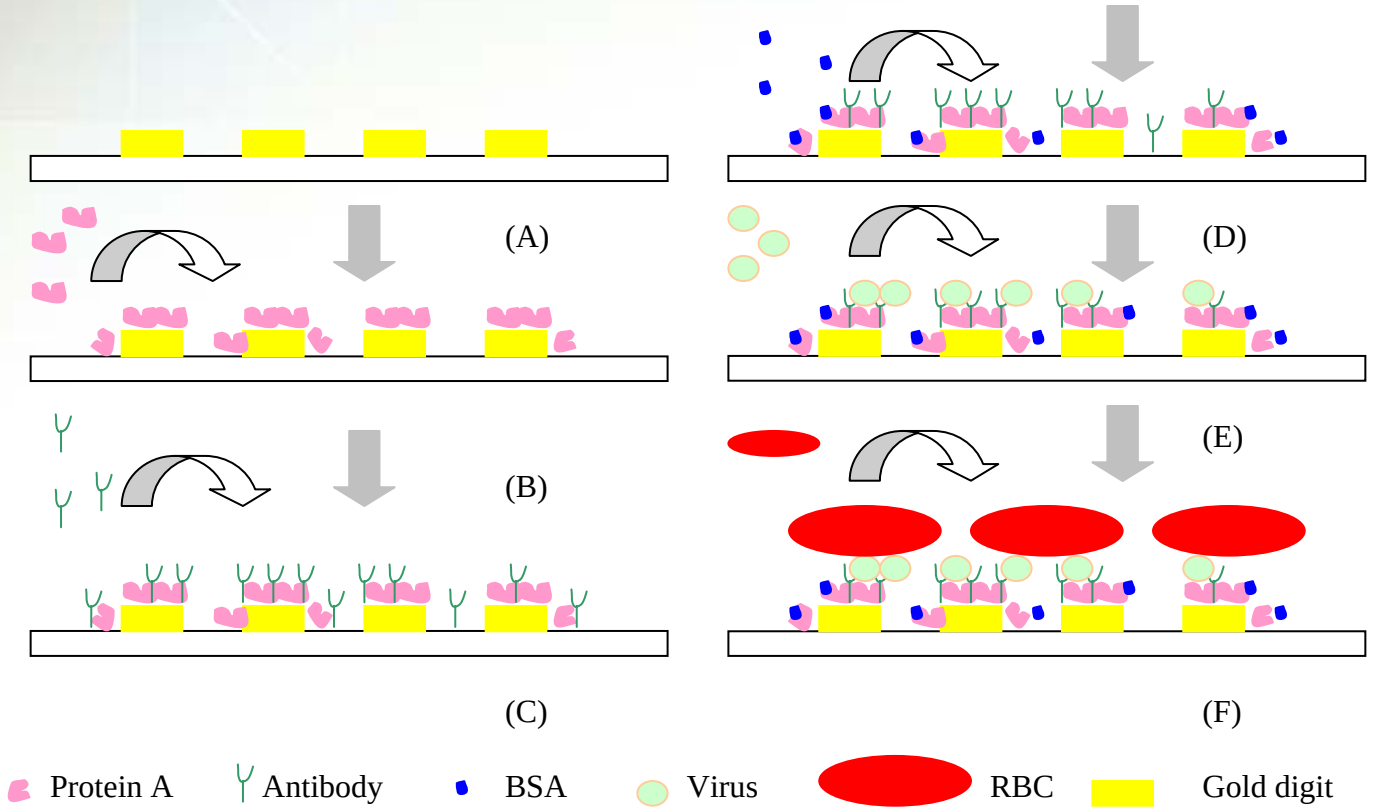
- *Experimental set up*

Experimental set up

IM-6 impedance analyzer (BAS, West Lafayette, IN)

Materials and methods

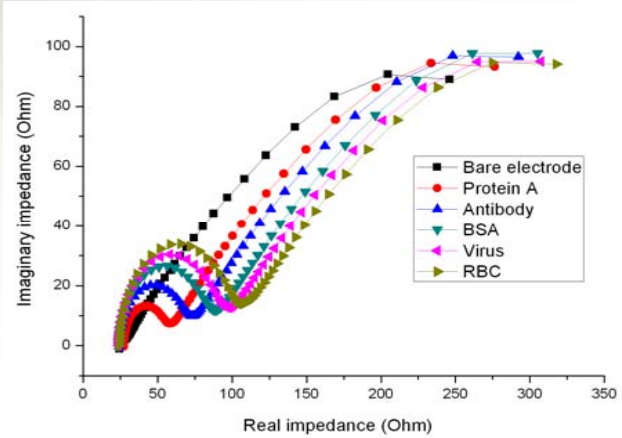
- *The procedure of viral detection*



Schematic diagram of experiment steps: (A) bare electrode; (B) modify the microelectrode with protein A; (C) immobilize antibodies onto the microelectrode; (D) apply BSA blocking; (E) capture AIV; (F) RBC binding

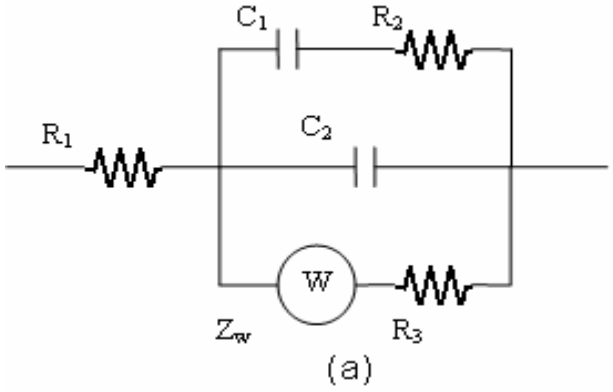
Results and discussion

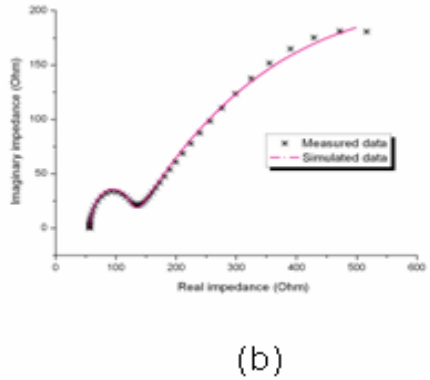
- *Typical sensorgram*



Nyquist diagram of electrochemical impedance spectra in the frequency range from 1 Hz to 1000 kHz. The Data points from left to right correspond to decreasing frequency. Amplitude voltage, 5 mV.

- *Equivalent circuit*

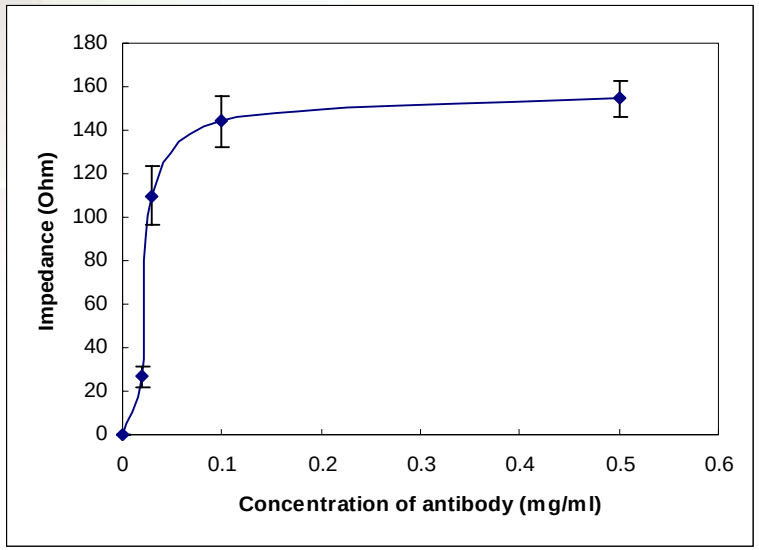




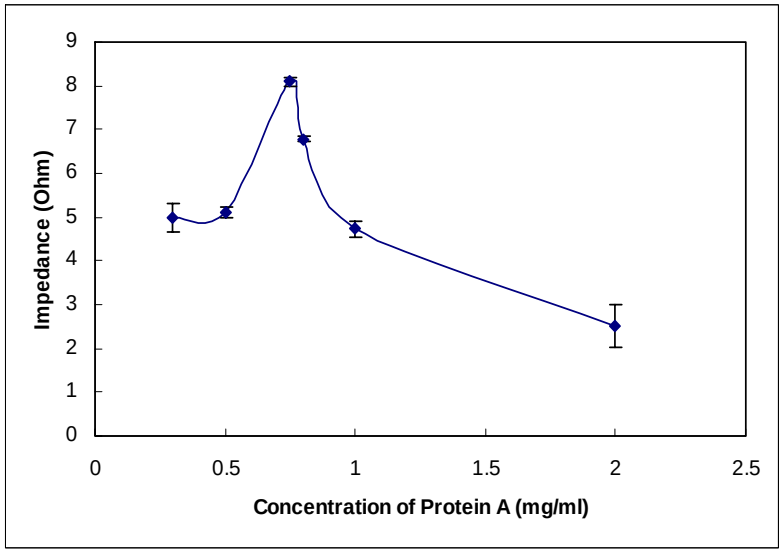
(a) Equivalent circuit, and (b) the simulation

Results and discussion

- *Optimization of IDA surface modification*
  - The concentration of protein A and antibody



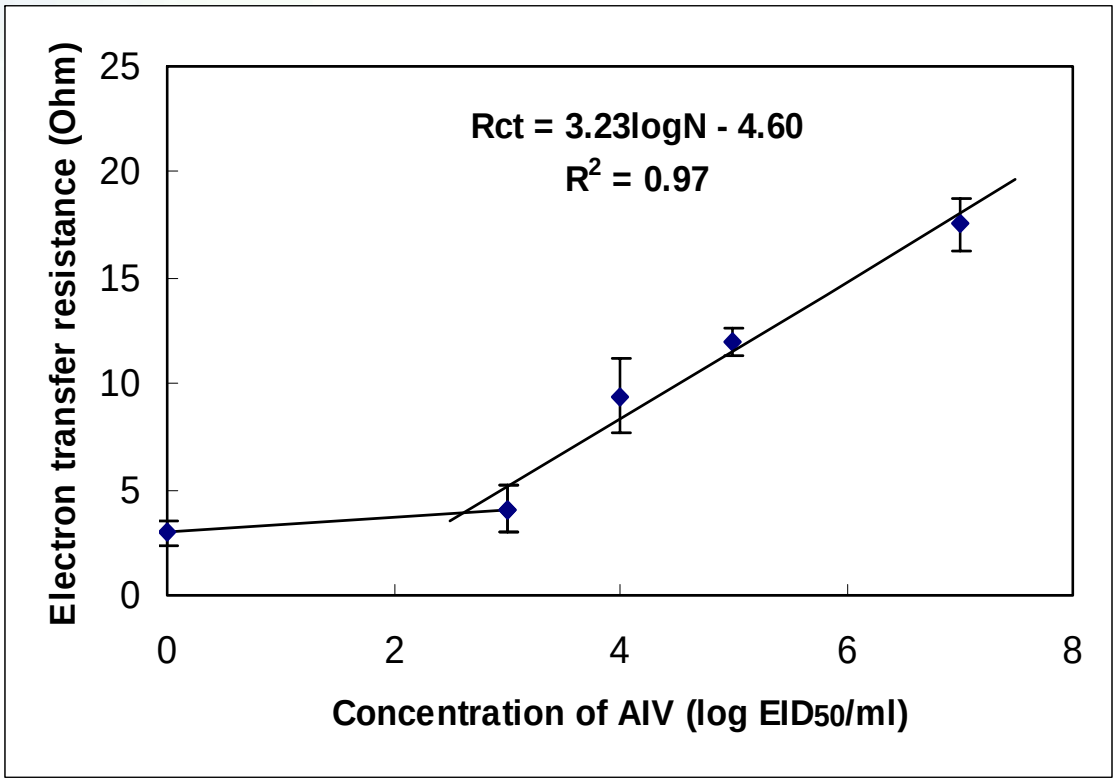
Relationship between the change of impedance and the concentration of antibody



Relationship between the change of impedance and the concentration of Protein A

Results and discussion

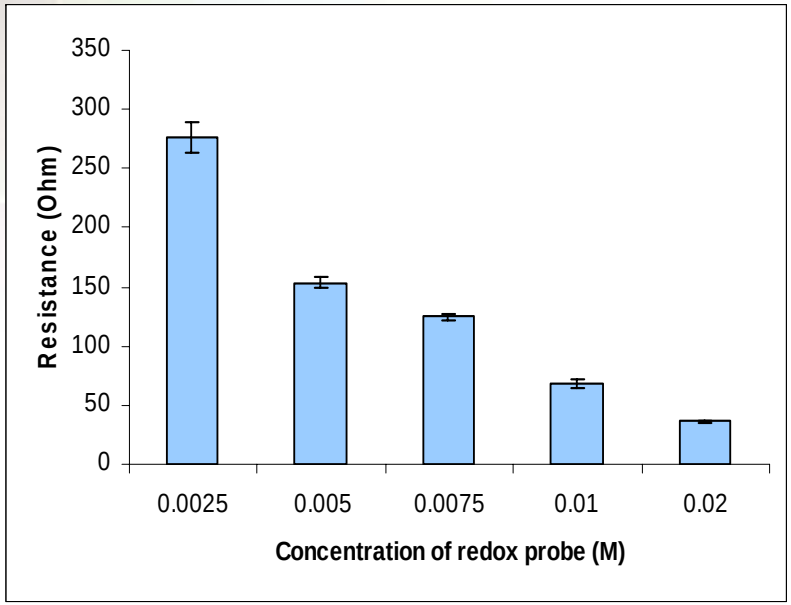
- *The detection limit*



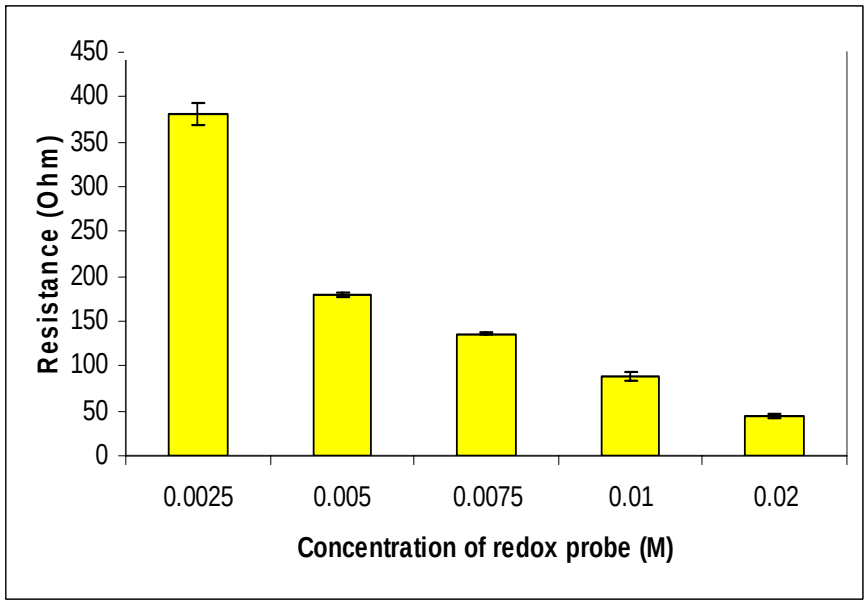
Linear relationship between the logarithmic value of the concentration of AIV and the change of electron-transfer resistance

Results and discussion

- *Optimization of impedance measurement*
  - The concentration of the redox probe



(a)

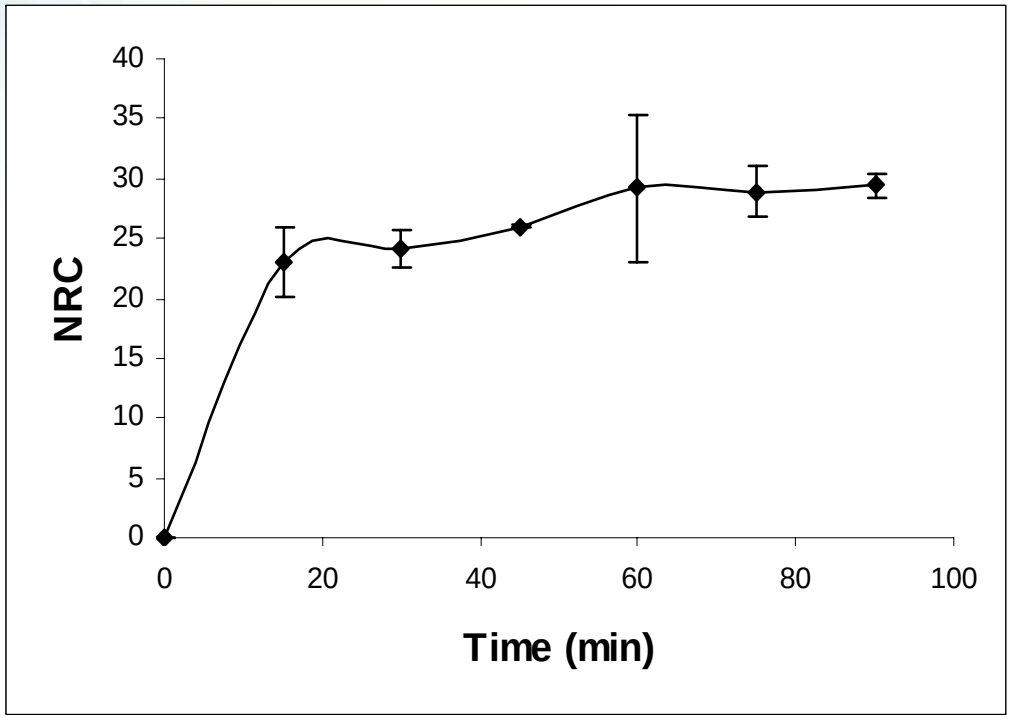


(b)

Electron transfer resistance versus concentration of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ (1:1) mixture: (a) detection of 10^5 EID_{50}/ml, and (b) detection of 10^3 EID_{50}/ml AIV

Results and discussion

- *The detection time*

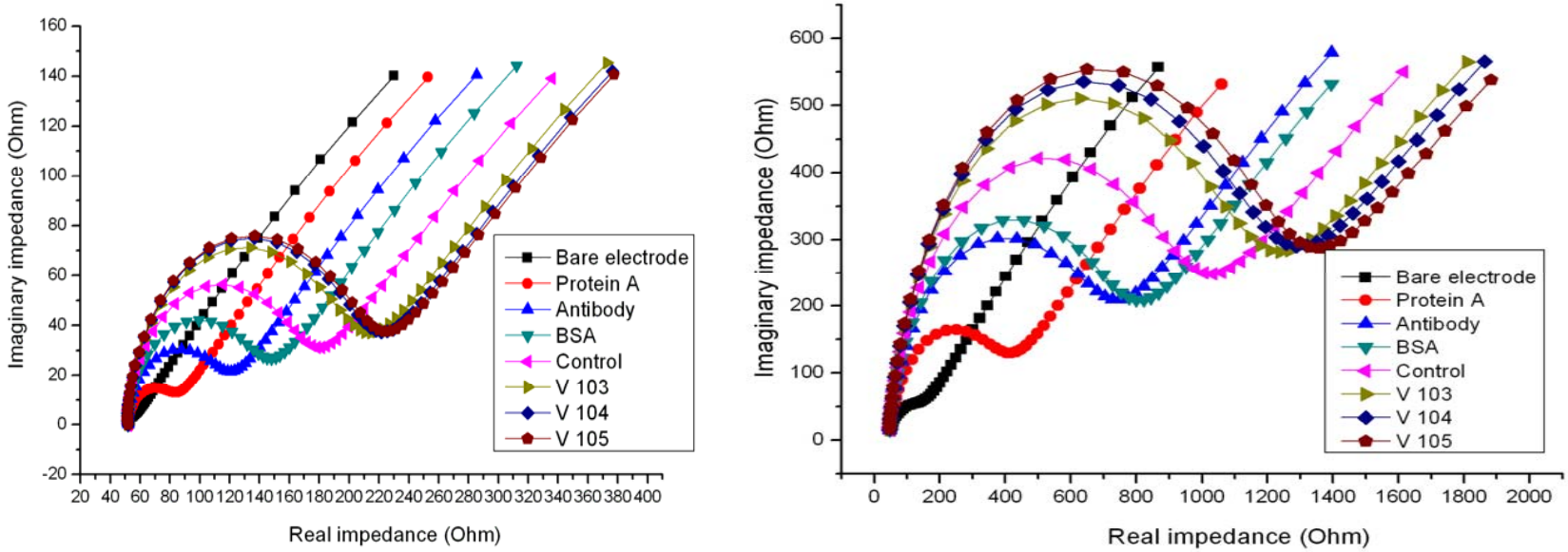


Relationship between the normalized change of electron transfer resistance (NRC) and detection time (the time for the virus incubation)

$NRC = (R_{virus} - R_{control})/R_{control}$

Results and discussion

- *The effect of the number and the size of electrodes*

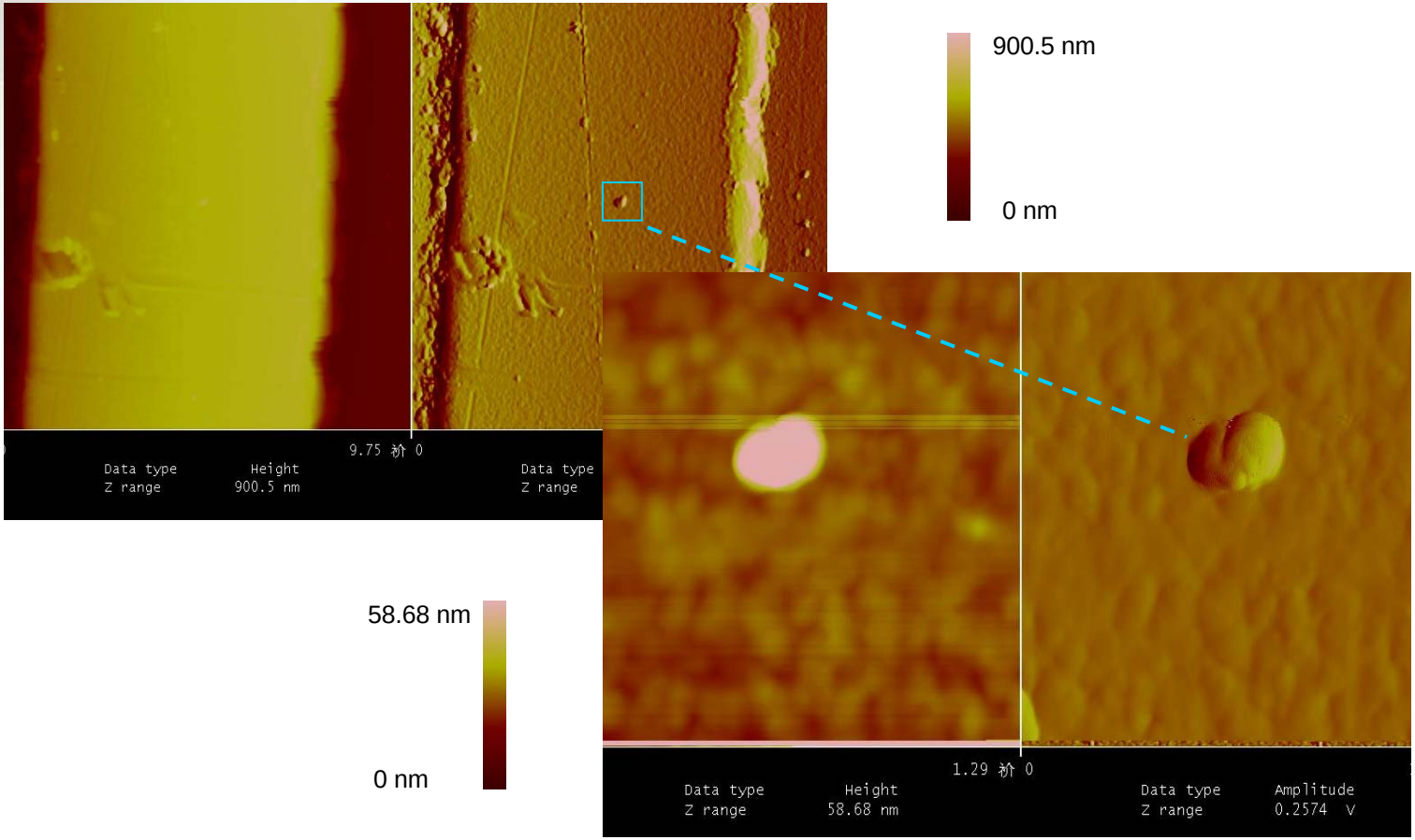


(a) (b)

Nyquist diagram of EIS of virus (10^3-10^5 EID_{50}/ml), in the presence of redox probe 0.005 M $[Fe(CN)_6]^{3-/4-}$ Data points from left to right correspond to decreasing frequency. Chip size: (a) 50-15-15, and (b)25-5-5

Results and discussion

- *AFM images of AIV on electrode*



AFM images of viruses captured on electrodes

Conclusions

- An IDA immunosensor was successfully applied in detection of AIV H5N1 based on impedance measurement.
- The limit of detection of 10^3 EID_{50}/ml with the detection time of within one hour can be achieved.
- A linear relationship between the electron transfer resistance (*Rct*) change and the logarithmic value of AIV concentration was found with R^2 value of 0.97.
- The performance of the IDA based immunosensor was improved by studying protein A modification, antibody immobilization, impedance measurement and et al.

Thank you!

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